Exhibit A

EXPRESS MAIL NO.: EM061 036 485US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Samulski et al.

Application No.: 08/475,470 Group Art Unit: 1809

Filed: June 7, 1995 Examiner: A. Nelson

For: ADENO-ASSOCIATED VIRUS VECTOR AND CIS-ACTING REGULATORY AND PROMOTER ELEMENTS CAPABLE OF EXPRESSING AT LEAST ONE GENE AND METHOD OF USING SAME FOR GENE THERAPY

Attorney Docket No.: 7639-077 (formerly 115132-4)

DECLARATION UNDER 37 CFR §1.132 BY RICHARD J. SAMULSKI

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, RICHARD JUDE SAMULSKI, do declare:

1. I am an inventor of the above-identified application.

2. I am an associate professor at the Department of Pharmacology at the University of North Carolina, with which I have been affiliated since June, 1993. I am experienced in the field of virology, particularly as it relates to adeno-associated virus (AAV) and the use of AAV for gene therapy. (See Curriculum Vitae, attached herewith as Exhibit A).

3. The invention disclosed in the above-identified application relates to recombinant adeno-associated virus

vectors for gene delivery and regulated tissue specific expression in a host. The recombinant adeno-associated vectors contain a mammalian gene of interest, genetically engineered adjacent to *cis*-acting regulatory and promoter elements, in such a way as to regulate expression in a tissue specific manner. The recombinant vectors can be used therapeutically to treat a variety of different genetic or acquired diseases.

4. Studies were conducted to determine whether recombinant AAV vectors could be used to efficiently transfer genes of therapeutic interest in an *in vivo* autologous bone marrow transplant setting. In particular, recombinant AAV vectors were used to transduce enriched primate hematopoietic progenitor cells which were then transplanted back into the primate host. The recombinant AAV vectors were genetically engineered to contain the neo^r gene.

5. Primate bone marrow (BM) was harvested and progenitor cells were enriched for by positive immunoselection for cells expressing the CD34+ antigen. $1\text{-}2 \times 10^7$ immunoselected cells from six animals were transduced with recombinant AAV virus for 6-8 hours in media, with or without, stem cell factor and interleukin-6 (CSF+/CSF-). Following incubation with recombinant AAV virus, the cells were washed and transplanted back into γ-irradiated primates.

6. Detection of transduced viral DNA in the transplanted primates was performed using a semi-quantitative

PCR assay for detection of the neo^r gene. As indicated in FIG. 1, viral DNA was detected in peripheral blood mononuclear cells (PB) and bone marrow (BM) from three of the six animals.

7. Viral DNA from the PB and BM of one animal (J352) was PCR positive at day 76. The animal remained positive until day 128 when it developed a bacterial infection and was euthanized. All the hematopoietic organs (liver, spleen, bone marrow, and thymus) taken from the animal at the time of death were positive for the presence of the transgene. Non-hematopoietic organs such as the brain, skeletal muscle and kidney were PCR negative for the transgene. The presence of the transgene was detectable in both flow cytometric purified populations of granulocyte and lymphocyte lineages as indicated in FIG.2.

8. Bone marrow from the PCR postive animal (J352) was tested for expression of the transduced neomycin gene. Bone marrow was harvested from the animal and plated in methylcellulose in the presence or absence of G418 (1.0 mg/ml). As indicated in FIG. 3, G418-resistant bone marrow colonies were obtained from the PCR positive animal (J352), while no colonies were obtained from the control animal. The data strongly supports that rAAV can not only infect primary hematopoietic cells, but also, express a functional protein.

9. The data presented indicates normal hematopoietic reconstitution of lethally irradiated primates following transplantation with recombinant AAV transduced CD34+ cells.

In addition, it was observed that recombinant AAV transduction levels can persist for up to at least three months (the duration of the experiment). *In vitro* colony forming unit (CFU) assays performed on the transplanted animals showed normal marrow reconstitution of neutrophils, erythrocytes, and platelets comparable to control animals indicating that transduction of recombinant AAV into bone marrow progenitor cells does not adversely affect reconstitution. The detection of the virally transferred transgene (neo^r) in both myeloid and lymphoid lineages further indicates the successful transduction of progenitor cells by recombinant AAV. Additionally, the ability of transduced cells to grow in the presence of G418 indicates successful expression of a functional protein.

10. I hereby declare further that all statement made herein by my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statement with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date_____

_____
Richard J. Samulski, Ph.D.

FIG. 1

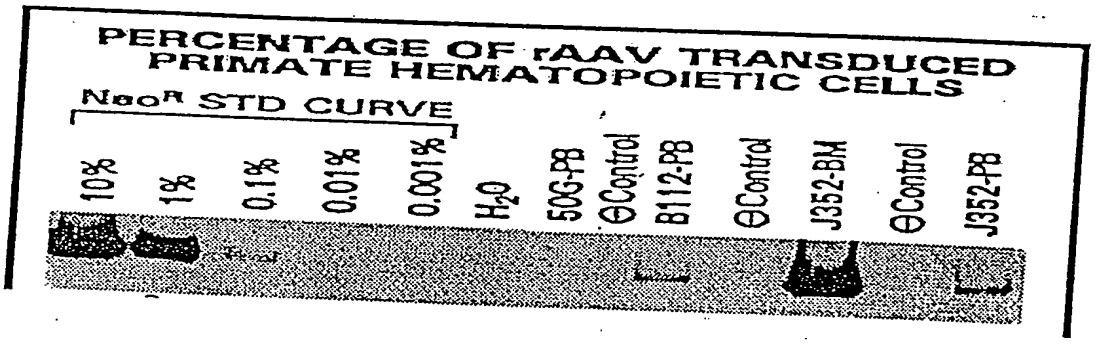
PERCENTAGE OF rAAV TRANSDUCED PRIMATE HEMATOPOIETIC CELLS
Neo^R STD CURVE
10%
1%
0.1%
0.01%
0.001%
H2O
50G-PB
Control
B112-PB
Control
J352-BM
Control
J352-PB

FIG. 2

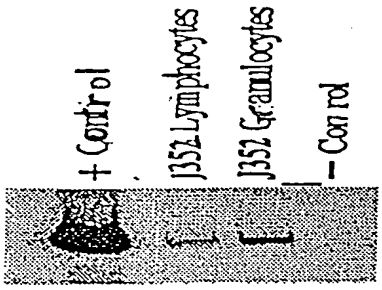

FIG. 3

Animal #I352	# of colonies/5x105 cells
-G418	60
+G418	10
Control Animal	
-G418	40
+G418	0